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MECHANISM OF STARFISH SPAWNING. IV. TENSION GENERATION IN THE OVARIAN WALL BY 1-METHYLADENINE AT THE TIME OF SPAWNING

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Abstract

Tension generation in the starfish ovaries at the time of spawning was investigated using an isometric tensiometer. Ovarian fragments containing oocytes gradually generated tension after 1-methyladenine application; before 1-methyladenine application the ovarian walls were not under strong tension. Isolated ovarian walls did not generate tension after 1-methyladenine treatment, but did after the application of jelly substance. As the hormone 1-methyladenine is known to act on oocytes surrounded by follicle envelopes to induce germinal vesicle breakdown, which inevitably results in the breakdown of follicle envelopes, it is concluded that (1) jelly substance acts directly to induce contraction of ovarian walls, (2) 1-methyladenine acts indirectly, contraction being caused as a result of the hormone's action on breakdown of germinal vesicles and follicle envelopes, after which jelly substance contacts the ovarian wall.

Introduction

Starfish spawning, including germinal vesicle breakdown and egg shedding, is caused by gonad-stimulating substance (GSS) secreted from nervous tissue (Chaet and McConnaughy, 1959; Chaet and Rose, 1961; Kanatani, 1964; Chaet, 1966, 1967; Kanatani and Ohguri, 1966). Since oocytes, adherently surrounded by follicle cells before spawning, are forced by contraction of ovarian walls through narrow gonopores, oocyte separation from follicle cells and contraction of ovarian walls are essential in shedding. Previous work has shown that GSS itself neither induces contractions in ovarian walls nor separates oocytes from follicles. On the other hand, 1-methyladenine (1-MeAde, produced by follicle cells under the influence of GSS) can cause oocyte-follicle separation following germinal vesicle breakdown. The chemical, a natural trigger of germinal vesicle breakdown, can by itself cause ovaries to spawn (Kanatani and Shirai, 1967; Schuetz and Biggers, 1967; Kanatani, 1967, 1969; Kanatani *et al.*, 1969; Schuetz, 1969; Hirai and Kanatani, 1971; Hirai *et al.*, 1973).

However, no work has dealt with contraction-inducing substance(s) within the ovary. The present study was carried out to examine whether 1-MeAde has contraction-inducing activity not, and how the ovarian wall contracts at spawning. We used an isometric tensiometer to measure contraction of the ovarian wall. The results showed that 1-MeAde causes contraction in ovarian fragments with oocytes, but not in isolated ovarian walls without oocytes. Isolated-ovarian-wall contraction

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Abbreviations: GSS: gonad-stimulating substance; 1-MeAde: 1-methyladenine; JF: jelly fraction.

was induced by jelly substance. The reason why eggs are shed concurrent with germinal vesicle breakdown in starfish under natural conditions is discussed.

Materials and Methods

Starfish (*Asterina pectinifera*) were collected in May in Kanagawa prefecture and kept in laboratoy aquaria with circulating cold seawater at the National Institute for Basic Biology. Ovaries were cut and washed with seawater. One branch of the ovary (about 5 mm long) was used in each measurement. Isolated ovarian walls without oocytes were prepared from ovarian fragments by cutting along their longitudinal axes with fine scissors and removing oocytes with follicle cells using fine forceps.

Artificial seawater was used (Jamarin or Calcium-free Jamarin, Jamarin Laboratory, Osaka). Jelly substance was obtained by dissolving jelly coats with HCl (pH 5.5) using eggs previously collected by treating ovaries for 1 h with 10^{-6} *M* 1-MeAde (Sigma). The egg suspension was kept for 1 h and occasionally agitated gently. The supernatant, containing dissolved jelly, was then centrifuged to remove debris and adjusted to pH 8 with NaOH. Concentrated jelly solution, obtained by centrifugation using Centri-flo C-25, was designated "jelly fraction" (JF). This process concentrated only molecules with molecular weights of more than about 25,000, and did not concentrate salt components. By this technique, about 1/100 of the volume of intact jelly was concentrated in JF (calculating the volume of intact jelly by measuring egg diameter, jelly coat thickness, and number of eggs).

Isometric contraction of ovarian fragments was measured with a horizontal-type tensiometer having a sensitivity of about 0.1 mg (Fig. 1) (Kamiya *et al.*, 1972; Yoshimoto and Kamiya, 1978). This enabled us to measure tension generated by a specimen submerged in a small quantity of solution (about 1 ml). A specimen was connected to the two fine glass rods of the tensiometer with a surgical adhesive agent, Aron alpha A (alkyl-α-cyanoacrylate, Sankyo Co.). Specimens were attached to the rods in the air (completed within 1 min). After attachment, the specimens were immersed in the appropriate test solution.

Results

Ovarian fragments with oocytes

Table I (upper part) shows that intact ovarian fragments, fixed on glass rods and treated with 1-MeAde, generated between 20 and 82 mg tension (mean 45 mg). Figure 2 shows a typical example.

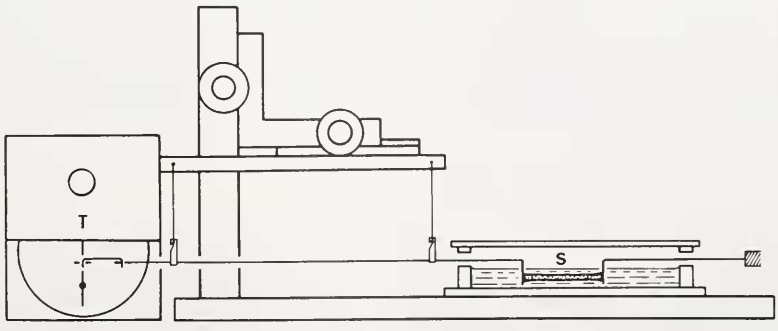

Figure 1. Scheme of a horizontal-type tensiometer. S, Specimen; T, Tension transducer.

TABLE I

Induction of tension in ovarian fragments. SE = standard error.

Exp. No.		Time lag (min)	Retained tension after pulling (mg)	Tension generation (mg)	Small oscillation	
					Amplitude (mg)	Period (s)
Intact	1	11	—	40	±1	60
	2	7	—	57	±1	60
Fragments	3	8	—	20	±1	60
	4	18	—	27	±1	80
	5	9	—	82	±1	48
Mean ± SE		11 ± 2	—	45 ± 11	±1	62 ± 5
Incised	1	15	6	125	±2	48
	2	16	15	130	±2	53
	3	16	6	80	±2	96
Fragments	4	16	9	105	±5	60
	5	5	9	91	±3	24
	6	10	13	73	±1	60
Mean ± SE		13 ± 2	10 ± 2	101 ± 10	±2.5	57 ± 10

After a time lag (between 1-MeAde application [final 10^{-5} *M*] to the beginning of tension increase) of mean 11 min, tension began to increase, reaching a high plateau 50–60 min after 1-MeAde application (21°C–23°C). This level was maintained for at least 2 h. Discharge of eggs from a cut end of the ovarian fragment usually began during the ascent to the high plateau. In addition to the tension generation, two types of oscillations were observed. One is designated small oscillation, with a period of about 1 min, and the other large oscillation, with a period of about 15 min (Fig. 2).

Next, ovarian fragments were simply cut along their longitudinal axes, leaving most oocytes still adhering to the walls. Since such incised fragments shortened from about 5 mm to 4.6 mm after cutting without 1-MeAde application, they were pulled to their original length by controlling the glass rod after the fragments were set on the tensiometer. Tension was generated just after this pulling, from several to more than a hundred 10 mgs, but then decreased rapidly (about 10 mg/min) and stayed at low levels (just over 10 mg) as shown in Table I (retained tension after pulling). After the fragments reached this resting state (about 20 min after pulling), 1-MeAde was added at a final concentration of 10^{-5} *M*, much as with intact ovarian fragments. As shown by Table I (lower part), the treated fragments generated strong tension (mean 101 mg) after a time lag (mean 13 min). Small oscillations were more obvious than in intact fragments, but large oscillations were not observed.

Contraction of isolated ovarian walls

Isolated ovarian walls without oocytes, prepared from intact fragments (not treated with 1-MeAde), were connected to the tensiometer and pulled, as were simply incised ones. As shown in Table II (upper part), they did not generate tension after 1-MeAde application, though 1-MeAde usually induced small oscillations. As these specimens generated sudden, temporary (but not retained) tension

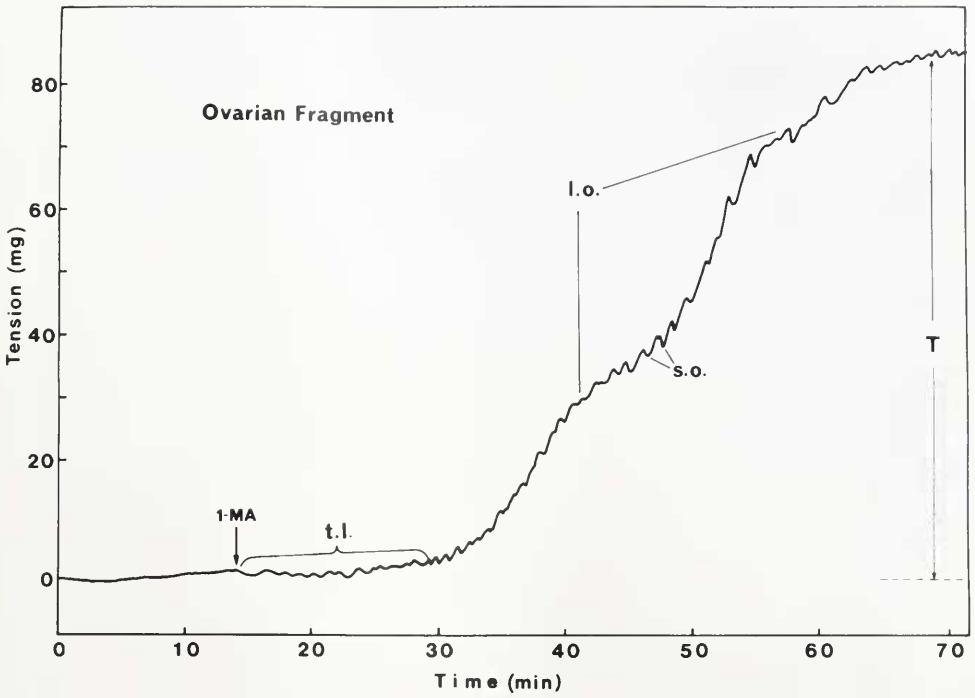

FIGURE 2. Induction of tension generation in intact ovarian fragments after 1-MeAde application. 1-MA, 1-MeAde application; T, tension generated; s.o., small oscillation in tension change; l.o., large oscillation in tension change; t.l., time lag.

on addition of KCl solution (10/9 *M*, one drop applied close to the specimen), the contractile structure had not been harmed during preparation. Figure 3 (upper left insertion) shows the tension change in specimens after application of KCl solution.

When isolated ovarian walls prepared from 10^{-5} *M* 1-MeAde treated (15 min) fragments were connected to the tensiometer, the retained tensions stayed at high levels (mean 27 mg) in the presence of 1-MeAde. This indicates that contraction actually had begun in ovarian walls treated with 1-MeAde.

Since the important difference between ovarian fragments and isolated walls was the presence or absence of oocytes, some intermediate substance(s) from oocytes seem to be involved in inducing contraction. We tested jelly substance, because jelly surrounds every oocyte and inevitably contacts the ovarian wall after breakdown of follicle envelopes from oocytes. The lower part of Table II shows that isolated walls generated tension after adding JF, with a mean time lag of 2 min (in this case the time lag was regarded as the period between JF application and beginning of tension increase). Small oscillations were much smaller than with 1-MeAde. (Fig. 3).

DISCUSSION

It was initially thought that the ovarian walls of intact fragments were originally under strong tension, corresponding to that generated in fragments to which 1-MeAde had been applied, because after incision the ovaries turned inside-out, with

TABLE II

Tension retention and generation in isolated ovarian walls.

Exp. No.	Retained tension after pulling (mg)	Time lag (min)	Tension generation (mg)	Small oscillation	
				Amplitude (mg)	Period (s)
1-MeAde application					
1	10	—	0	±1	80
2	20	—	0	±1	96
3	11	—	0	±0	irregular
4	25	—	0	±2	40
5	10	—	0	±0	irregular
Mean ± S.E.	15 ± 3	—	0	±1	72 ± 17
JF application					
1	9	2	35*	not detectable	
2	18	2	28*	not detectable	
3	9	2	29	not detectable	
4	22	2	27	not detectable	
5	11	3	18	not detectable	
6	10	2	21	not detectable	
Mean ± S.E.	13 ± 2	2 ± 0.2	26 ± 2	not detectable.	

* JF and 1-MeAde ($10^{-5}M$) added.

the mass of oocytes lying on the external surface, and the length of the fragment shortened (Kanatani, 1967). However, as shown in Table I (retained tension after pulling), these ovarian walls did not retain the strong tension displayed just after they were pulled. Therefore, it is unlikely that ovarian walls are originally under strong tension. However, when the incised ovarian fragments were tested without pulling (no tension displayed before 1-MeAde application), they generated less tension after 1-MeAde application than did pulled fragments. Smooth muscle generally displays a well-defined length-tension relationship: if the original length of

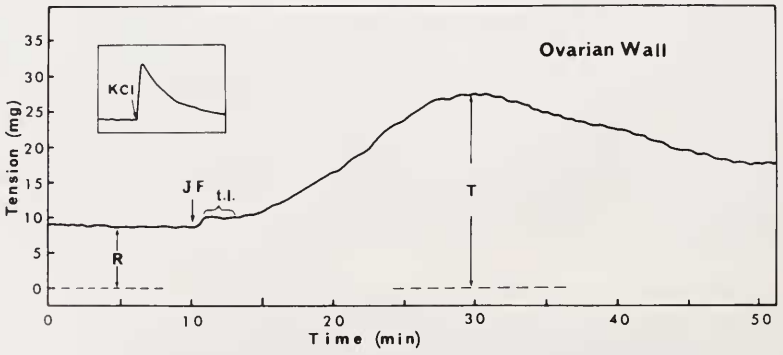

FIGURE 3. Induction of tension generation in isolated ovarian walls prepared from intact ovarian fragments after "jelly fraction" (JF) application. JF, JF application; R, Retained tension; T, tension generated; t.l., time lag; KCl, KCl application.

the specimen is increased or reduced, its ability to generate tension changes. If smooth muscle is shortened, its ability to generate tension is reduced (Millman, 1964). Thus ovarian walls at their original length are more able to generate strong tension than after shortening.

We experimented using isolated ovarian walls because the purpose of the present study was not to investigate the mechanism of smooth muscle contraction, but to test 1-MeAde action as the direct inducer of ovarian contraction. As shown in Table II, isolated walls did not generate tension after 1-MeAde application, though these specimens displayed phasic contraction after stimulation with KCl (Twarog, 1976), as described in Results. However, these specimens generated strong tension after JF was added (Table II, lower part). Therefore, it can be concluded that JF contains the substance inducing contraction of isolated ovarian walls.

Fully grown ovaries are filled with numerous oocytes with a jelly coat surrounded by follicle cells adhering to each other. At spawning, the oocytes have already lost the follicle envelopes and are freely movable, and the ovarian wall contracts. GSS (released from nervous tissue) causes ovaries to spawn within about 30 min after its application. However, GSS itself does not possess oocyte-separating activity affecting oocyte-follicle cell adhesion, or contraction-inducing activity on ovarian walls. With respect to spawning GSS only acts on follicle cells to produce 1-MeAde (Kanatani, 1973, 1979; Kanatani and Shirai, 1969, 1970). On the other hand, 1-MeAde, produced by follicle cells within a very short period after GSS application, by itself causes ovaries to spawn. GSS or 1-MeAde initiate spawning in approximately the same time, about 30 min. Moreover, 1-MeAde has oocyte-separating activity: When oocytes still surrounded by follicle cells were immersed in seawater containing 1-MeAde, germinal vesicle breakdown took place within 15–20 min and inevitably resulted in the breakdown of follicle envelopes. Although we have not yet investigated the actual process of how the follicle envelopes break down, we know that 1-MeAde acts on the separation of the oocyte from the follicle cell. This follicle envelope breakdown subsequently leads to direct contact between ovarian walls and the jelly layer of oocytes within ovaries. In summary, jelly substance acts directly on contraction of ovarian walls, while 1-MeAde acts indirectly, causing contraction as a result of the action of the hormone on germinal-vesicle and follicle-envelope breakdown, after which jelly substance contacts the ovarian wall.

Although follicle cells and oocytes can be experimentally separated in calcium-free seawater, in this condition ovarian fragments never contract, even if they contact jelly (Kanatani, 1967; Schuetz and Biggers, 1968; Kanatani and Shirai, 1969, 1970). Therefore, when jelly substance causes ovarian walls to contract, calcium must be available for certain processes, undoubtedly including the actual contraction of muscle.

Egg spawning in individuals (egg shedding from gonopores) usually occurs within 1 h after 1-MeAde injection into the coelomic cavity. Microscopic observations show that the gonopore, closed until egg discharge begins, is opened by eggs. Since the tension generated will have almost reached its high plateau by the time the gonopore opens, the force that keeps the gonopore closed (which we have not measured) must be less than that of the high plateau level in the ovarian wall. And since egg discharge continues until almost all eggs are expelled, the mechanism which keeps the force in the ovarian walls stronger than that at the gonopore is essential for the completion of spawning.

Ovarian wall contraction induced by jelly components may be a part of the mechanism which induces ovarian contraction in the limited area around oocytes which have undergone germinal vesicle breakdown: in an intact ovary during spawn-

ing, as more and more oocytes mature, the number of limited areas contracting within the ovarian wall would thus increase until the total tension was greater than the force at the gonopore. This tension would be greatest once all, or almost all oocytes were mature. Thus, only mature oocytes might be spawned.

Contraction-inducing substances common to both female and male may exist, because 1-MeAde, produced by interstitial cells of the testes (Kubota *et al.*, 1977), also causes shedding of spermatozoa after a certain time lag (less than 1 h).

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